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BIOACTIVITY OF SEAWEEDS AGAINST SOIL-BORNE PLANT PATHOGENS

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ABSTRACT

Ethanolic extract of seventeen species of seaweeds were tested against juveniles of *Meloïdogyne javanica*. Five seaweeds, *Sargassum binderi*, *Stokeyia indica*, *Caulerpa taxifolia*, *Codium iyengarii*, and *Rhizoclonium implexum* caused 100% juvenile mortality at 10 mg/mL after 48 hours. Five more seaweeds (*Padina pavonia*, *Spatoglossum asperum*, *Spatoglossum variable*, *Botryocladia leptopoda*, and *Solieria robusta* also caused larval mortality more than 50% at 10 mg/mL. *Stokeyia indica* and *Soliera robusta* also caused larval mortality more than 50% at the dose level of 1 mg/mL. Ethanolic extracts were also tested against root infecting fungi *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani*, and *F. oxysporum*. *Spatoglossum asperum* and *Spatoglossum variable* inhibited radial growth of *M. phaseolina*, *R. solani*, and *F. solani* in vitro when used at 6 mg/disc. *Stoechospermum marginatum* and *Codium iyengarii* also inhibited the growth of *F. solani* at the same concentration.

KEY WORDS: seaweeds, nematicide, fungicide

INTRODUCTION

Plant disease-causing organisms produce extensive damage to crop plants and adversely affect the agricultural economy of a country. Among the plant pathogens, soil-borne root infecting fungi [*viz.*, *Macrophomina phaseolina* (Tassi) Goid., *Rhizoctonia solani* Kuhn, *Fusarium solani* (Mart.) Appel. & Wollenw. emend. Snyd. & Hans, and *F. oxysporum* Schlecht.] and root knot nematode (*Meliodogyne* spp.) attack roots of plants, limit nutrient uptake by the plant, and produce root rot-root knot disease complex, resulting in the death of the plant. Such conditions were found to be very common in agricultural fields of Pakistan (Ehteshamul-Haque & Ghaffar 1994;

Maqbool 1992). Seaweeds are generally used for enhancement of plant growth (Atzmon *et al.* 1994). Seaweeds are also known to reduce the fecundity of the root knot nematode on tomato (Whapham *et al.* 1994). Marine algae have been reported to possess a wide range of bioactive properties (Hoppe & Levering 1982). They showed antioxidative (Yan *et al.* 1998) and antitumor activity (Zhuang *et al.* 1995). Liquid concentrations of the brown alga *Ecklonia maxima* (Osbeck) Papenfuss significantly reduced root knot infestation and increased growth of tomato (Featomby-Smith & Standen 1983). Antimicrobial and cytotoxic activities of seaweed have been reported (Hodgson 1984; Ara *et al.* 1999). Ara *et al.* (1996a) reported nematicidal activity of some seaweeds from Pakistan. However, a detailed study on the effect of seaweeds on soil-borne root infecting fungi and root knot nematode is lacking. The present report describes in vitro bioactivity of seaweeds against the root infecting fungi *Macrophomina phaseolina*, *R. solani*, *F. solani*, and *F. oxysporum*, as well as the root knot nematode *Meloidogyne javanica* (Treub) Chitwood.

MATERIALS AND METHODS

Brown, green, and red algae [viz., *Dictyota dichotoma* (Huds.) Lamour, *Iyengaria stellata* (Borg.) Borg., *Padina pavonia* (L.) Lamour, *Sargassum binderi*, *Sargassum variegatum*, *Spatoglossum asperum* J. Ag., *Spatoglossum variable* Fig. & D.E. Notar., *Stoechospermum marginatum* (C. Ag.) Kutz., *Stokeyia indica* Thivy & Doshi, (brown); *Caulerpa racemosa* (Forsk.) J. Ag., *Caulerpa taxifolia* (Vahl.) C. Ag., *Codium iyengarii* Borg., *Rhizocloniun implexum* (Dillw.) Kutz., *Ulva lactuca* L., (green); *Botryocladia leptopoda* (J. Ag.) Kylin, *Halymenia porphroides* C. Ag., *Sciania indica*, and *Solieria robusta* (Greville) Kylin (red)] were collected from Buleji Beach, Paradise Point, and Pacha Beach, Karachi, Pakistan in different seasons at low tide. Different species of seaweeds exposed on sand and rocks were collected in plastic bags and brought to the laboratory. Each species of seaweed was washed under tap water and dried under shade. The seaweeds were then powdered in an electric blander and stored in polyethylene bags at room temperature until used.

Dry powder of seaweeds (500 g each) were extracted three times with ethanol (4× volume) for one week. Extracts were pooled, filtered through cotton wool and concentrated to dryness on rotary vacuum evaporator and weighed. Antimicrobial activity of ethanolic extract of seaweeds was determined by the method used by Ahmad *et al.* (1986). The method was modified using a dilution of 200 mg/mL of crude extract of seaweed prepared in ethanol. The sterilized thick filter paper discs (5 mm) were impregnated with these dilutions at 2, 4, and 6 mg/disc and dried. Discs were placed at different peripheral positions of petri dishes containing Czepak's Dox agar (pH 7.2). Discs impregnated with only ethanol served as negative control, while benomyl (10 yg/disc) served as positive control. A 5 mm disc of actively growing culture of test fungi (*Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani*, and *F. oxysporum*) was inoculated in the center of the petri dishes. Each treatment was replicated three times and plates were incubated at 28° C. Zones of inhibition produced were recorded daily.

Table 1. *In vitro* growth inhibition of *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani,* and *F. oxysporum* by the ethanolic extract of seaweed.

	Zone of inhibition (mm)			
Seaweeds	*M. phaseolina*	*R. solani*	*F. solani*	*F. oxysporum*
Control	0	0	0	0
Standard (benomyle)				
10 yg/disc	13	7	14	15
PHAEOPHYTA				
Dictyota dichotoma				
2 mg	0	0	0	0
4 mg	0	0	0	0
6 mg	0	0	0	0
Iyengaria stellata				
2 mg	0	0	0	0
4 mg	0	0	0	0
6 mg	0	0	0	0
Padina pavonia				
2 mg	0	0	0	0
4 mg	0	0	0	0
6 mg	0	0	0	0
Sargassum binderi				
2 mg	0	0	0	0
4 mg	0	0	0	0
6 mg	0	0	0	0
Sargassum variegatum				
2 mg	0	0	0	0
4 mg	0	0	0	0
6 mg	0	0	0	0
Spatoglossum asperum				
2 mg	0	0	0	0
4 mg	0	0	0	0
6 mg	2	2	5	0
Spatoglossum variabile				
2 mg	0	0	0	0
4 mg	0	0	0	0
6 mg	*	2	5	0
Stoechospermum marginatum				
2 mg	0	0	0	0
4 mg	0	0	0	0
6 mg	0	0	3.5	0

Table 1. (continued)

Seaweeds	Zone of inhibition (mm)			
	M. phaseolina	*R. solani*	*F. solani*	*F. oxysporum*
CHLOROPHYTA				
Caulerpa racemosa				
2 mg	0	0	0	0
4 mg	0	0	0	0
6 mg	0	0	0	0
Caulerpa taxifolia				
2 mg	0	0	0	0
4 mg	0	0	0	0
6 mg	0	0	0	0
Codium iyengarii				
2 mg	0	0	0	0
4 mg	0	0	0	0
6 mg	0	0	2	0
Rhizoclonium implexum				
2 mg	0	0	0	0
4 mg	0	0	0	0
6 mg	0	0	0	0
Ulva lactuca				
2 mg	0	0	0	0
4 mg	0	0	0	0
6 mg	0	0	0	0
RHODOPHYTA				
Botryocladia leptopoda				
2 mg	0	0	0	NT
4 mg	0	0	0	NT
6 mg	0	0	0	NT
Halymenia porphroides				
2 mg	0	0	NT	0
4 mg	0	0	NT	0
6 mg	0	0	NT	0
Sciania indica				
2 mg	0	0	0	NT
4 mg	0	0	0	NT
6 mg	0	0	0	NT
Solieria robusta				
2 mg	0	0	0	NT
4 mg	0	0	0	NT
6 mg	0	0	0	NT

*inhibited, but no zone was produced
NT=not tested

Table 2. *In vitro* mortality of *Meloidogyne javanica* juveniles at different concentration of ethanolic extract of seaweeds after 48 hours.

	Juveniles mortality (%) at various concentrations (mg/mL)			
Seaweeds	0.01	0.1	1.0	10.0
Control	0	0	0	0
PHAEOPHYTA				
Dictyota dichotoma	0	0	6	10
Iyengaria stellata	4	3	7	36
Padina pavonia	3	0	27	71
Sargassum binderi	2	6	18	100
Sargassum variegatum	3	10	60	100
Spatoglossum asperum	0	0	16	53
Spatoglossum variabile	0	29	33	77
Stoechospermum marginatum	3	12	17	83
Stokeyia indica	0	3	75	100
CHLOROPHYTA				
Caulerpa racemosa	0	0	21	30
Caulerpa taxifolia	0	3	5	100
Codium iyengarii	3	24	43	100
Rhizoclonium implexum	5	7	13	100
Ulva lactuca	0	6	14	28
RHODOPHYTA				
Botryocladia leptopoda	3	4	7	50
Halymenia porphroides	6	12	17	20
Sciania indica	8	8	11	26
Solieria robusta	0	44	56	98

$LSD_{0.05}$ seaweed=6.4, concentration=2.9

The nematicidal activity of seaweed was determined by the method used by Ara *et al.* (1997) using 0.01, 0.1, 1.0, and 10.0 mg/mL concentrations of seaweed extract were prepared in ethanol. Two mL of each concentration was transferred to a small watch glass and left for 48 hours to evaporate the organic solvent. Twenty hand-picked second stage juveniles of *Meloidogyne javanica* were placed in each glass, containing 2 mL glass distilled water. A watch glass without extract served as control. Each treatment was replicated three times. The number of juveniles that were killed after 48 hours were recorded using a stereomicroscope. Data were subjected to analysis of variance (ANOVA) followed by least significant difference (LSD) (Gomez & Gomez 1984).

RESULTS AND DISCUSSION

Of the seventeen seaweed species tested against root infecting fungi, *Spatoglossum asperum* and *Spatoglossum variable* inhibited the growth of *Macrophomina phaseolina, Rhizoctonia solani,* and *Fusarium solani,* at 6 mg/disc. *Spatoglossum asperum* produced 2, 2, and 5 mm zones against *M. phaseolina, R. solani,* and *F. solani* respectively, while *Spatoglossum variable* produced 2 and 5 mm zones against *R. solani* and *F. solani* respectively. *Stoechospermum marginatum* and *Codium iyengarii* produced 3.5 and 2 mm zones against *F. solani* respectively (Table 1).

Of the seaweed species tested *Sargassum binderi, Stokeyia indica, Caulerpa taxifolia, Codium iyengarii,* and *Rhizoclonium implexum* caused 100% mortality of juveniles after 48 hours at the concentration of 10 mg/mL, whereas *Padina pavonia, Spatoglossum asperum, Spatoglossum variable, Stoechospermum marginatum, Botryocladia leptopoda, J. capillacea,* and *Solieria robusta* showed more than 50% mortality at the dose level of 1 mg/mL (Table 2).

In the present study, some seaweeds showed nematicidal and fungicidal activity. Growth inhibition of several bacteria and fungi by seaweed has been reported (Shyamali *et al.* 1982). Out of 30 seaweeds belonging to brown, red, and green algae tested, most of which showed antibacterial and hemolytic activity (Rao *et al.* 1991). Febles *et al.* (1995) reported antimicrobial activity of Canary Island species of Phaeophyta and Chlorophyta. Shaikh *et al.* (1990) isolated four diterpenoides from *Stoechospermum marginatum* which exhibited antibacterial and antifungal activities. There are reports that seaweed extracts derived from *Ascophyllum nodosum* (Linnaeus) Le Jolis reduced *Radopholus similis* infection on citrus (Tarjan 1977). Soil amendment with brown seaweeds, *Stoechospermum marginatum* and *Sargassum tenerrimum* significantly reduced gall formation on mungbean plants caused by *Meloidogyne javanica*, and enhanced plant growth (Siddiqui *et al.* 1998). Ara *et al.* (1996b) reported that soil amendment with *Sargassum* species significantly reduced infection of *Macrophomina phaseolina, Rhizoctonia solani,* and *Fusarium solani* on sunflower. Use of brown seaweed *Stoechospermum marginatum* significantly reduced gall formation on okra caused by *Meloidogyne javanica* (Ehteshamul-Haque *et al.* 1996). In the present study, *Spatoglossum asperum* showed growth inhibition of *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* and larval mortality of *Meloidogyne javanica. Spatoglossum asperum* could be exploited for the isolation of nematicidal and fungicidal compounds. *Stokeyia indica* and *Solieria robusta* also

showed significant nematicidal activity at 1 mg/mL and could also be exploited for the isolation of nematicidal compounds. Seaweeds which showed promising results could also be used as an organic amendment for the control of root infecting fungi and root knot nematode which will result in increased crop productivity.

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